

Antimicrobial activity of the Coelomic fluid of Earthworm against disease causing bacteria

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Abstract - Earthworms are first annulated eucoelomate organism having complete digestive system, closed circulatory system with haemoglobin in the plasma to carry oxygen and carbon di oxide. Many biological activities are shown by the coelomic fluid of the earthworm *Eisenia fetida*. A study was made to monitor antimicrobial effect of the coelomic fluid using the zone of inhibition technique against bacterial strains of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Shigella flexneri*, *Enterococcus faecalis*. The best inhibitory effect of *Eisenia fetida* coelomic fluid on the growth of *Shigella flexneri* observed was 12mm and that on *Staphylococcus aureus* was 9mm and on *Enterococcus faecalis* was 7mm, whereas the zone of inhibition observed was minimum 3mm against *Pseudomonas aeruginosa*.

Keywords - earthworm, coelomic fluid, bacteria, microbial, pathogens

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INTRODUCTION

There have been many studies on the presence of antimicrobial substances in annelids since the first bacteriolytic molecules were identified as lysozyme like molecules, which were found active only against gram-positive bacteria (Jolles and Zuili 1960; Schubert and Messner 1971; Lassegues 1986). The coelomic fluid of the earthworm, *Eisenia fetida* was found to possess an antimicrobial activity against pathogenic bacteria namely; *Aeromonas hydrophila* Gram-negative [(Chester) Stanier] and Gram-positive *Bacillus megaterium* (de Bary) by Valembos *et al* (1982). Later, Milochau *et al* (1997) reported that immune response of earthworms includes both cellular and humoral components. Cooper *et al*, 2001, reported that the coelomocytes of earthworm react to the presence of pathogens by phagocytosis, encapsulation/brown body formation and N K cells activity. Invertebrates have developed innate immune mechanism that detects pathogens by recognizing conserved molecular patterns. Molecules responsible for the recognition of foreign material have been named as pattern recognition proteins (PRP's) (Medzhitov and Janeway, 1997) because the host's primitive effector cells would recognize molecular pattern rather than particular structure of the invading microorganisms. This study was conducted with an aim to detect the antimicrobial activity of the coelomic fluid of *Eisenia fetida* against pathogenic bacteria like *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Shigella flexneri*, *Enterococcus faecalis*.

MATERIALS AND METHODS

Collection of industrial effluents and hospital effluent

Industrial effluents collected from paper, stone and textile industries located at RIICO Industrial Area, Sanganer Jaipur and Hospital waste collected from SDMH Hospital Jaipur were sprinkled on specific cultures of earthworm to maintain moisture.

Collection and culture of earthworms

Earthworm, *Eisenia fetida*, collected from Department of Soil Science and Agricultural Chemistry (SK Rajasthan Agricultural University) and Agricultural Research station, Durgapura Jaipur were maintained in earthen pots containing a mixture of soil and cow dung in 2:1 ratio. Optimum temperature was maintained by constant sprinkling water in the culture pots.

Collection and culture of bacterial strains

Four different types of bacteria namely *Shigella flexneri*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Staphylococcus aureus* were purchased from IMTECH Chandigarh, India and they were transferred separately on freshly prepared nutrient agar medium at 35°C to 37°C for 24 hours to obtain active culture. Each bacterium was transferred separately to fresh nutrient broth from this active culture and incubated at 35°C to 37°C for

24 hours to get active culture suspension. Nutrient broth and agar were purchased from HIMEDIA.

Experimental Design

Earthworms from each experimental culture were washed with distilled water and kept on filter paper for drying. Coelomic fluid was collected directly from the body cavity of earthworms without causing any harm to them by cold shock, heat shock and electric shock. The fluid released was pipette out using a sterilized pipette with a fine nozzle. Thus, the pure coelomic fluid was obtained which can be used for different biological investigations (Kale, 2006). Collected coelomic fluid was centrifuged at 3000 rpm for 15 minutes to sediment debris and larger particles. Supernatant was carefully removed and collected in sterile Eppendorf tubes and antibacterial tests were conducted using the supernatant. Sterilized blotting paper was impregnated with 25µl of the fraction to be tested and placed on agar dishes inoculated with bacterial strains. The bacterial species were separately cultured in the nutrient broths and were separately spread plated on nutrient agar plates. Then, the impregnated disc with 25µl coelomic fluid was inserted and incubated upright at 37°C in an incubator for 24 hours. The antimicrobial activity of the fractions was measured in terms of mm zone of inhibition.

RESULTS

The anti-microbial activity of the coelomic fluid of *Eisenia fetida* against pathogenic bacteria was measured and recorded in mm inhibition zone formation around the coelomic fluid impregnated discs. The best bactericidal activity of earthworm coelomic fluid was measured and recorded against bacteria *Shigella flexneri* was 12mm and that against *Staphylococcus aureus* was 9mm, against *Enterococcus faecalis* was 7mm and least zone of inhibition was noted against *Pseudomonas aeruginosa* i.e., 3mm. The above results indicate that the coelomic fluid of species of earthworm is species specific in antibacterial activity. No zone of inhibition was recorded for the coelomic fluid of earthworms treated with textile effluents as all the worms were dead and fragmented into pieces due to the extensive use of dyes and chemicals in the textile industries resulting in highly polluted effluent.

DISCUSSION

Our results tally with those observed by Popovic *et al* 1996, who discussed that the coelomic fluid of earthworm exhibit antimicrobial activity because of their innate immunity and they detect microorganism by recognizing conserved molecular pattern. Our folklore and modern medicines report the use of animal tissues and fluids like placenta, snake and bee poison, sea and river hydroceles for treatment of several human diseases. The fibrin can be dissolved by the tissue fluid of earthworm as reported by Darwin and also Many Japanese researchers.

Our results indicate that coelomic fluid fractions of *Eisenia fetida* could decrease growth of pathogenic bacteria. The antimicrobial activity existing in the ECF was suggested to arise from yellow pigment (riboflavin), a lysozyme like activity and fetidins (Hirigoyenberry *et al* 1991; Lassalle *et al* 1988). Maya *et al* (2005) have reported that a glycolipoproteins mixture G-90, obtained from tissue omogenate of earthworm *Eisenia fetida* and showed antibacterial activity in vivo and in vitro in various concentrations of growth on non pathogenic and facultative pathogenic bacteria. Rejnek (1991) and Valembois *et al* (1993) demonstrated the antibacterial activity of the earthworms and that they were assisted by chloragocytes. These observations are made on zone of inhibition in vitro. Further research on the presence of different antimicrobial factors in the coelomic fluid of earthworms is required which will help the medical world to know the medicinal efficacy of the coelomic fluids of indigenous species of earthworms.

SUMMARY AND CONCLUSION

Since ancient times earthworms are known for their medicinal value. Earthworms have closed circulatory system containing blood and the coelomic fluid. It has both haemocytes and coelomocytes which play an important role in fighting against pathogens. This quality of coelomic fluid initiated the curiosity for investigating the immunological and antibacterial activity. Thus, in conclusion it can be said that coelomic fluid of *Eisenia fetida* shows antibacterial properties against pathogenic bacteria. However, further studies are required for finding many other antibacterial molecules in coelomic fluid.

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